

Appl. No. 09/888,320
Amdt. dated 7/27/04
Amendment under 37 CFR 1.116 Expedited Procedure
Examining Group 1634

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Amendments to the Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Currently amended) A method of determining the ability of a *Mycobacterium tuberculosis* bacterium to oxidize
 - (a) ~~ethionamide, thiacetazone or thiocarlide~~, ethionamide, said method comprising detecting a mutation in an *EtaA* gene (SEQ ID NO:1) in said bacterium, which mutated gene encodes an amino acid sequence which differs from that of SEQ ID NO:2 by
 - (a) (i) a frameshift mutation selected from the group consisting of: a deletion at position 65, an addition at position ~~567~~ 557, and an addition at position 811, or
 - (b) (ii) a single nucleotide polymorphism which causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P,
 - (b) thiacetazone, said method comprising detecting a mutation in the *EtaA* gene (SEQ ID NO:1) in said bacterium, which mutated gene encodes an amino acid sequence which differs from that of SEQ ID NO:2 by
 - (i) a frameshift mutation selected from the group consisting of: a deletion at position 65 and an addition at position 811, or
 - (ii) a single nucleotide polymorphism which causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, T342K, and A381P, or
 - (c) thiocarlide, said method comprising detecting a mutation in the *EtaA* gene (SEQ ID NO:1) in said bacterium, which mutated gene encodes an amino acid sequence which differs from that of SEQ ID NO:2 by
 - (i) a frameshift mutation selected from the group consisting of: a deletion at position 65 and an addition at position 811, or
 - (ii) a single nucleotide polymorphism which causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, and A381P,

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wherein detection of the mutation is indicative of decreased ability to oxidize ethionamide, thiacetazone or thiocarlide, respectively.

2. (Canceled)

3. (Original) The method of claim 1, wherein the mutation is a single nucleotide polymorphism which causes an amino acid substitution in an amino acid sequence encoded by said EtaA gene compared to an amino acid sequence of SEQ ID NO:2.

4. (Canceled)

5. (Original) A method of claim 1 wherein the mutation is detected by
(a) amplifying the EtaA gene, or a portion thereof containing the mutation, with a set of primers to provide an amplified product,
(b) sequencing the amplified product to obtain a sequence, and
(c) comparing the sequence of the amplified product with the sequence of a wild-type EtaA gene (SEQ ID NO:1) or portion thereof,
wherein a difference between the sequence of the amplified product and the sequence of the wild-type EtaA gene or portion thereof indicates the presence of a mutation.

6-7. Canceled.

8. (Original) A method of claim 5, wherein said amplification is by polymerase chain reaction.

9. (Original) A method of claim 1, wherein said mutation is detected by hybridizing DNA from said bacterium to a test nucleic acid under stringent conditions.

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10. (Original) A method of claim 9, wherein either said DNA from said bacterium or said test nucleic acid is immobilized on a solid support.

11. (Original) A method of claim 1, wherein said mutation is detected by
(a) subjecting said EtaA gene to digestion by restriction enzymes,
(b) separating the resulting restriction products to form a pattern of restriction fragment lengths, and
(c) comparing the pattern of restriction fragment lengths to a pattern of restriction fragment lengths formed by subjecting a known EtaA gene to the same restriction enzymes.

12 - 20. (Canceled)

21. (Currently amended) A method of screening an individual for a *Mycobacterium tuberculosis* bacterium resistant to treatment by ~~ethionamide, thioacetazone or thiocarlide~~,

(a) ethionamide, comprising
(a) (i) obtaining a biological sample containing said bacterium from said individual, and
(b) (ii) detecting a mutation in an EtaA gene (SEQ ID NO:1) in said bacterium, which mutated gene encodes an amino acid sequence which differs from that of SEQ ID NO:2, wherein said mutation in said EtaA gene is selected from the group consisting of
(i) (A) a frameshift mutation consisting of a deletion at position 65, an addition at position 567 557, or an addition at position 811, and (ii) (B) a single nucleotide polymorphism which causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P,

(b) thiacetazone, comprising

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(i) obtaining a biological sample containing said bacterium from said individual,

and

(ii) detecting a mutation in an EtaA gene (SEQ ID NO:1) in said bacterium, which mutated gene encodes an amino acid sequence which differs from that of SEQ ID NO:2, wherein said mutation in said EtaA gene is selected from the group consisting of
(A) a frameshift mutation consisting of a deletion at position 65 or an addition at position 811,
and (B) a single nucleotide polymorphism which causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, T342K, and A381P, or

(c) thiocarlide, comprising

(i) obtaining a biological sample containing said bacterium from said individual,

and

(ii) detecting a mutation in an EtaA gene (SEQ ID NO:1) in said bacterium, which mutated gene encodes an amino acid sequence which differs from that of SEQ ID NO:2, wherein said mutation in said EtaA gene is selected from the group consisting of
(A) a frameshift mutation consisting of a deletion at position 65 or an addition at position 811,
and (B) a single nucleotide polymorphism which causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, and A381P,

wherein detection of the mutation is indicative said bacterium is resistant to treatment by
ethionamide, thiacetazone or thiocarlide, respectively.

22. (Original) A method of claim 21, wherein the mutation is detected by

(a) amplifying the EtaA gene with a set of primers to provide an amplified
product,

(b) sequencing the amplified product to obtain a sequence, and

(c) comparing the sequence of the amplified product with the sequence of
a wild-type EtaA gene (SEQ ID NO:1),

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wherein a difference between the sequence of the amplified product and the sequence of the wild-type EtaA gene indicates the presence of a mutation.

23-34. Canceled.

35. (Currently amended) The method of claim 34 1, wherein the mutation is a frameshift mutation selected from the group consisting of: a deletion at position 65, an addition at position ~~567~~ 557, and an addition at position 811.

36. (Canceled)

37. (Currently amended) The method of claim 34 3, wherein the single nucleotide polymorphism causes an amino acid substitution selected from the group consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.

38-48. (Canceled)